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Regulation of inosine monophosphate dehydrogenase type I and type II isoforms in human lymphocytes

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Abstract

The enzyme inosine monophosphate dehydrogenase (IMPDH) catalyzes the rate-limiting step in the *de novo* biosynthesis of guanine nucleotides. Inhibition of IMPDH leads to immunosuppression by decreasing guanine nucleotides that are required for the proliferation of lymphocytes. IMPDH activity is mediated by two highly conserved isoforms, type I and type II. We have characterized the mRNA and protein expression of the two isoforms in a variety of human tissues, peripheral blood mononuclear cells (PBMCs), and selected cell lines to investigate their regulation. Type I mRNA was expressed in most tissues with high expression in PBMCs and low expression in thymus. IMPDH type II transcript was also detected in most tissues with low expression in spleen and PBMCs. In PBMCs, induction of both type I and type II mRNAs was observed within 12 hr of mitogenic stimulation. Using type-selective IMPDH antibodies, an increase in the levels of type I and type II proteins was observed after mitogenic stimulation. The effect of two IMPDH inhibitors, MPA and VX-497, was investigated on the expression of type I and type II isoforms. VX-497 is an orally bioavailable, potent and reversible inhibitor of IMPDH, with broad applicability in many viral and immune system-mediated diseases. MPA and VX-497 inhibit both isoforms of IMPDH *in vitro*. Prolonged treatment of lymphocytes with either VX-497 or MPA did not lead to an increase in type I or type II IMPDH protein levels. These results are discussed in the context of IMPDH being a target for immunosuppressive, anti-viral and anti-cancer therapy.

Keywords: Lymphocytes; IMPDH; Gene regulation; Mycophenolic acid; VX-497; Immune

1. Introduction

IMPDH catalyzes the rate-limiting step in the *de novo* biosynthesis of guanine nucleotides that are required for the proliferation of lymphocytes. It is encoded by two distinct genes, type I and type II, located on two different chromosomes [1–3]. Both cDNAs contain open reading frames corresponding to 514 amino acids and proteins of 56 kDa. The two isoforms share 85% identity at the amino acid level and have virtually identical kinetic properties [4–6]. Despite the high level of identity, the two isoforms are not mutually redundant. Homozygous deletion of IMPDH type II gene results in embryonic lethality at day 9 of gestation in mice [7], indicating that the activity of IMPDH type I enzyme is unable to substitute for the activity of IMPDH type II enzyme during early embryonic development. Deletion of the type I gene has a milder phenotype [8].

The three-dimensional crystal structure of IMPDH type II isoform demonstrates IMPDH as a homotetramer with subunit molecular masses in the 56–58 kDa range [9,10]. Increase in IMPDH enzyme activity has been correlated with increased cellular proliferation and transformation [11,12]. Thus, IMPDH has been an attractive target for immunosuppressive, anti-viral and anti-tumor therapy for more than two decades. The drug MMF (or CellCept®), the ester pro-drug of MPA, is approved for the prevention of acute graft rejection in kidney, heart and liver transplantation [13] in combination with steroids and cyclosporine A (CsA). Mizoribine (Bredinin®) and ribavirin (Virazole®, Rebetol®) are nucleoside analogs, which following intracellular phosphorylation are competitive

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Abbreviations: IMPDH, inosine-5′-monophosphate dehydrogenase; PBMCs, peripheral blood mononuclear cells; MPA, mycophenolic acid; MMF, mycophenolate mofetil; IC_{50}, concentration required to inhibit 50% response; PMA, phorbol-12-myristate-13-acetate; PWM, pokeweed mitogen.

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IMPDH inhibitors [6,14,15]. Mizoribine is approved in Japan for multiple indications including prevention of rejection after renal transplantation, idiopathic glomerulonephritis, lupus nephritis, and rheumatoid arthritis [15]. Ribavirin is used as an inhaled anti-viral agent for treatment of respiratory syncytial virus (RSV) and, orally in combination with interferon-α, for the treatment of chronic hepatitis C viral (HCV) infection [14]. Antineoplastic and anti-viral activities of other nucleoside and NAD analogs, such as tiazofurin for the treatment of chronic myelogenous leukemia (CML) have been described [12], but these are not yet approved for clinical use. We have designed several non-nucleoside, uncompetitive, potent and selective IMPDH inhibitors using structure-based drug design [6]. Of these, VX-497 is currently being investigated in a Phase II trial in combination with pegylated interferon and ribavirin for the treatment of hepatitis C [16,17]. VX-148 is being evaluated for treatment of psoriasis [18] and VX-944 for hematological malignancies [19].

In this study, we have examined the effect of two potent and specific IMPDH inhibitors, MPA and VX-497, on the expression level of the two IMPDH isoforms. Since both MPA and VX-497 inhibit IMPDH type I and type II enzymes almost equipotently with K_i values of 7–10 nM [17,20], it is important to establish whether both enzymes are valid therapeutic targets. While both isoforms are expressed in most tissues, previous studies have generally implicated changes in expression of IMPDH type II gene as being closely linked with cellular growth and differentiation [1,11]. A single 2.3-kb type II transcript is the predominant transcript in transformed cells and is selectively upregulated in proliferating cells [11]. The same transcript is observed to be downregulated upon induction of terminal differentiation of cells which also correlates with a decrease in their proliferation [11]. The type I mRNA species has been described to be constitutively expressed during cell proliferation and differentiation [1]. However, Dayton *et al.* [21] reported an increase in the expression of both type I and type II mRNAs in human lymphocytes stimulated with the T cell mitogens, PMA and Ionomycin. We have confirmed these results and extended them to examine the regulation of individual IMPDH isoforms at the mRNA as well as the protein level.

2. Materials and methods

2.1. Reagents

Most cell culture reagents, unless specified otherwise, were obtained from Gibco-BRL or JRH Biologicals. Complete RPMI was prepared by adding up to a final of 10% fetal bovine serum (FBS), 55 μM β-mercaptoethanol, 50 units/mL penicillin, 50 μg/mL streptomycin, 300 μg/mL L-glutamine and 10 mM HEPES, pH 7.5, to RPMI 1640. Stock solutions of PMA (Calbiochem), Ionomycin (Calbiochem), PWM (Sigma Chemical Co.), were prepared in FBS-free RPMI and stored at −20°. *Staphylococcal* protein A immobilized on Sepharose CL-4B (SPAS, Pharmacia) was freshly prepared on the day of the assay in complete RPMI. VX-497 (synthesized at Vertex Pharmaceuticals), and MPA (Sigma), were dissolved in DMSO at a concentration of 20 mM and stored at −20°.

2.2. Isolation and stimulation of peripheral blood mononuclear cells

Human venous blood was drawn from healthy volunteers using heparin as an anti-coagulant, or obtained as leukopaks from Massachusetts General Hospital. PBMCs were isolated from blood by centrifugation over Ficoll-paque gradient or CPT tubes (Becton-Dickinson) using standard conditions. PBMCs were harvested, washed and resuspended in complete RPMI, counted and diluted to 1×10^6 cells/mL [17]. PBMCs were stimulated with PMA (10 ng/mL) and Ionomycin (250 ng/mL), or PWM (25 μg/mL).

2.3. Cell lines

All cell lines were obtained from American Tissue Culture Collection (ATCC), and cultured in the growth medium specified by ATCC. The cell lines investigated were Jurkat human acute T cell leukemia, L1210 mouse lymphocytic leukemia, Raji human B cell Burkitt's lymphoma and WI38 human diploid lung fibroblast cell line.

2.4. Northern blot analysis

Human and murine tissue blots for Northern analysis were purchased from Clontech. Total cellular RNA was prepared using Trizol reagent from Gibco-BRL as per manufacturer's recommendations. The RNA marker ladder was obtained from Gibco-BRL. The filters were hybridized with cDNA probes specific for IMPDH type I or type II genes (see below), and then exposed to either X-ray film or Fuji phosphorimager for quantification of radioactivity in individual bands. To normalize blots for uniform loading of RNA, gels were either stained with ethidium-bromide or the blots were hybridized with β-actin (Clontech), or 18S rRNA (Clontech) probes. The probes were stripped according to the instructions included in the ExpressHyb hybridization solution (Clontech).

Human type I or type II cDNAs were restriction digested with *Msc*I, separated on an agarose gel and the fragment corresponding to 425 bp, spanning exons 3–7, was isolated and purified using Geneclean (Bio101) [3]. The fragments were radiolabeled with [^{32}P]-ATP using Random Primer Labeling kit as per supplier's instructions (Gibco-BRL). The probes were purified using Chromo Spin columns (Clontech). ExpressHyb hybridization solution, hybridization and washing conditions were as specified by the

manufacturer (Clontech). Experiments were performed at least two to three times, with a representative blot being shown.

2.5. Antiserum and antibodies

Peptides for generating pan-IMPDH, IMPDH type I- and type II-specific rabbit antisera were synthesized at Vertex Pharmaceuticals Inc. The sequence of pan-IMPDH peptide [(C)VHGLHSYEKRLY], corresponds to amino acids 503–513 in human IMPDH. The sequence of type I peptide [(C)AEKDHTTLLSE], and type II peptide (KEEEHDCFLE), corresponds to amino acids 167–176 in the respective human IMPDH proteins [2,3]. Keyhole limpet hemocyanin (KLH) conjugated peptides were prepared using Pierce Reduce-Immune and the Pierce Imject (maleimide) activation kits. Rabbits were immunized with water in oil preparations of the KLH-peptides, first in complete and subsequently incomplete Freund's adjuvant. The antisera were affinity-purified using the cognate peptides with the Cysteine at the N-terminus coupled to a preactivated resin (SulfoLink Coupling Gel, Pierce). The antisera were passed over the resin equilibrated in phosphate-buffered saline (PBS). The bound antibodies were eluted with glycine buffer (0.1 M glycine/HCl, pH 2.5) according to the manufacturer's instructions. The affinity purified antibodies were buffered with 1 M Tris, pH 9.5, concentrated to a protein concentration of 0.24 mg/mL of type I, 0.28 mg/mL of type II and 0.46 mg/mL of pan-IMPDH antibody. The antibodies were stored at -20° until further use. Recombinant human type I and type II IMPDH proteins were expressed and purified [22], and used to determine the specificity of each antibody.

2.6. Western blot analysis

Total cellular protein was isolated using RIPA buffer (20 mM Tris–HCl, pH 7.2, 150 mM NaCl, supplemented with 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, 1 mM benzamide, 2 mM DTT, 2 mM PMSF and benzonase) spiked with protease inhibitors (1% aprotinin, 10 μg/mL pepstatin, 10 μg/mL leupeptin). Typically for PBMCs, 1×10^8 cells were lysed in 0.5 mL of RIPA buffer. Lysates were normalized for protein concentration using BCA protein assay (Pierce), using BSA as standard. Equal protein (20–40 μg), was loaded in each lane of a 4–20% denaturing gel (Novex), and transferred to nylon membranes as recommended by Novex. The blots were incubated with affinity-purified antibodies at dilutions of 1:100 and 1:500 for the type I and type II antibodies, respectively. The membranes were developed using Pierce SuperSignal ECL kit after treating the membranes with 1:5,000–1:10,000 dilutions of the secondary HRP conjugated anti-rabbit Ig (Amersham). Typically 20 ng of recombinant type I, or 40 ng of recombinant type II, protein was loaded per lane to serve as a reference. Molecular weights were estimated using the Kaleidoscope (BioRad) protein marker.

3. Results

3.1. Expression of IMPDH type I and II mRNAs in tissue samples

The steady-state levels of IMPDH type I and type II mRNAs in a variety of adult human tissues are shown in Fig. 1. A single transcript of 2.5 kb corresponding to type I mRNA is expressed in most tissues, with very high expression in resting PBMCs, spleen, lymph node and bone marrow in human (Fig. 1, top panel). The lowest levels of expression of type I mRNA are observed in human thymus, small intestine, ovary and mouse testis. Multiple bands are observed in the type I blot for spleen, lymph node and peripheral lymphocytes, similar to results described previously in human PBMCs [21]. The 2 kb IMPDH type II transcript is also detected in most tissues with highest expression observed in human ovary, small intestine and colon (Fig. 1, middle panel). Expression of type II IMPDH is low in spleen, and in contrast to type I, type II is low in resting PBMCs (Fig. 1, middle panel). Both IMPDH isoforms are also detectable at embryonic days 7–17 in mice, suggesting a role for each isoform during normal embryonic development (data not shown).

3.2. Induction of IMPDH type I and II mRNAs in mitogen-stimulated PBMCs

In PBMCs stimulated with PMA and Ionomycin, induction of type I mRNA is observed within 12 hr of mitogenic stimulation (Fig. 2, top panel). Three transcripts of 3.5, 2.5 and 2 kb are observed, and all three appear to be coordinately regulated. Stimulated IMPDH type I mRNA peaks around 24 hr, with some decrease observed by 48 and 72 hr. Type II mRNA is also induced in response to stimulation, with a single transcript of 2 kb being observed 12 hr after stimulation (Fig. 2, middle panel). Unlike type I, IMPDH type II mRNA stays elevated up to 48 hr, with a decrease evident only at 72 hr. In several independent experiments, no significant type I or type II mRNA signals were detected in unstimulated PBMCs. Hence, both type I and type II mRNAs are inducible in normal human lymphocytes.

3.3. Effect of IMPDH inhibitors on induction of individual isoform in activated lymphocytes

The effects of two IMPDH inhibitors, MPA and VX-497, were investigated on the expression of type I and type II genes. MPA and VX-497 inhibit both isoforms of IMPDH with K_i values of 7–10 nM [4,17,20]. Both VX-497 and MPA dose-dependently block the proliferation of human lymphocytes after stimulation with T- or B-cell mitogens

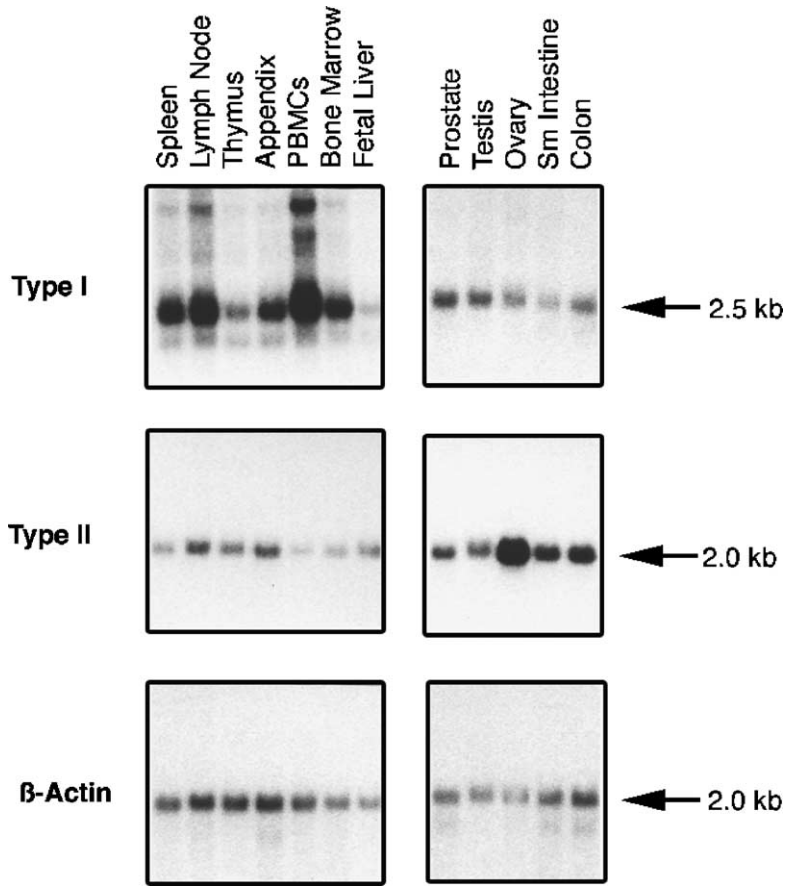

Fig. 1. Expression pattern of IMPDH type I and II mRNAs in human and murine tissues. Tissue blots (Clontech) were hybridized with cDNA probes for IMPDH type I (top panel), type II (middle panel), or β-actin genes (bottom panel).

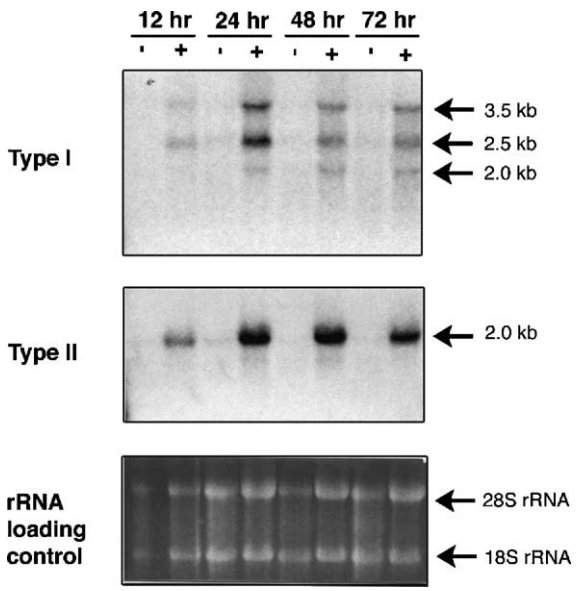

Fig. 2. Time course of induction of IMPDH type I and II genes in stimulated PBMCs. Total RNA was isolated from unstimulated PBMCs (−), or PBMCs stimulated (+) for 12, 24, 48, or 72 hr with 10 ng/mL PMA and 250 ng/mL Ionomycin. The filters were hybridized with cDNA probes for IMPDH type I (top panel) or type II (middle panel) genes. The gel was stained with ethidium bromide to visualize RNA loading (bottom panel).

with IC_{50} and IC_{90} values of approximately 100 and 300 nM, respectively [17,20]. A clinically relevant and achievable concentration of 1 μM VX-497 or MPA was selected for subsequent studies to achieve complete inhibition of proliferation. Treatment of human PBMCs with up to 1 μM VX-497 or MPA does not lead to any significant alteration in IMPDH type I or type II mRNA levels induced in response to mitogenic stimulation (Fig. 3A). This is the case for lymphocytes activated with PMA and Ionomycin (Fig. 3A), as well as stimulation with the lectin PWM (Fig. 3B), which activates both T and B cells. PWM also stimulates both type I and type II mRNAs (Fig. 3B); induction of type I mRNA appears to be comparable or higher than that of type II mRNA in these experiments.

3.4. Expression of IMPDH mRNAs in immortalized cell lines

In rapidly proliferating, transformed cell lines, different levels of type I and II mRNAs are observed (Fig. 4). In Raji B cells, the level of type II mRNA appears several-fold higher than type I mRNA, whereas in the L1210 murine leukemia cell line expression of both types is low. In WI38 human fibroblast cell line, both type I and type II mRNAs

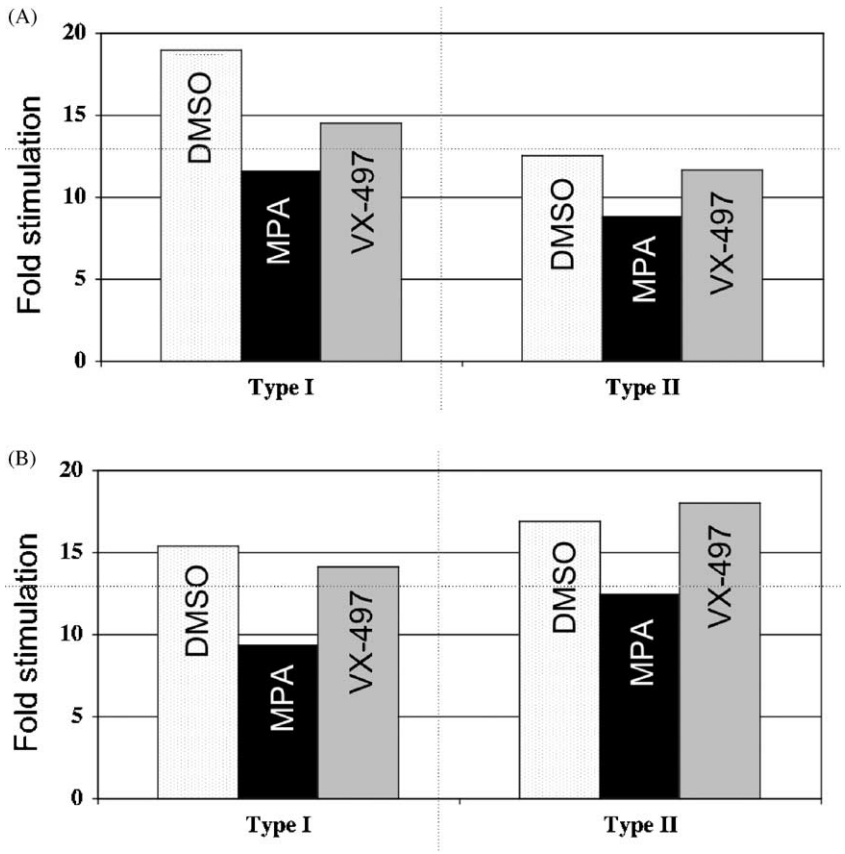

Fig. 3. Treatment of PBMCs with MPA or VX-497 does not alter the induction of IMPDH mRNAs significantly. Total RNA was isolated from unstimulated PBMCs, or PBMCs stimulated for 24, 48, or 72 hr with 10 ng/mL PMA and 250 ng/mL Ionomycin (A), or 25 μg/mL pokeweed mitogen (B). DMSO control, 1 μM MPA or VX-497 was included during the stimulation as indicated. The filters were hybridized with cDNA probes for IMPDH type I and type II genes. The bands were quantified and expressed as fold-induction over unstimulated levels. The values shown in figure represent average values at 24 hr calculated from two independent experiments. No significant effect of VX-497 or MPA was observed up to 72 hr. For example the average induction at 72 hr for type I mRNA was 16-fold in presence of MPA and 12-fold with VX-497.

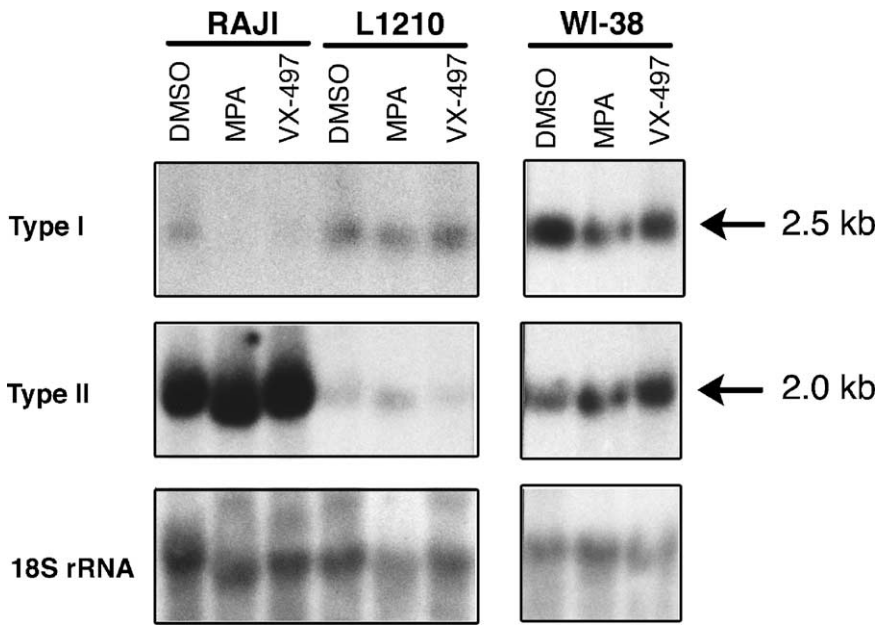

Fig. 4. Treatment of lymphoid or fibroblast cell lines with MPA or VX-497 does not alter IMPDH mRNA levels. Total RNA was isolated from Raji, L1210 or WI38 cells treated with 0.1% DMSO, 1 μM MPA or VX-497 for 72 hr. The blots were hybridized with IMPDH type I (top panel), IMPDH type II (middle panel), or 18S rRNA (bottom panel), probes.

are detected at similar levels. The 2.5 kb transcript is the major type I species observed in these cell types. Both MPA and VX-497 effectively block cell proliferation with IC_{50} values of 298 and 645 nM, respectively in Raji B cells, 359 and 449 nM, respectively in L1210 leukemia cells and >20,000 nM in WI38 fibroblast cells [17]. As observed in normal human lymphocytes, treatment of Raji, L1210 or WI38 cells with 1 μM VX-497 or MPA for up to 72 hr does not appear to cause any alteration in the steady-state levels of either isoform.

3.5. Characterization of IMPDH type I- and type II-specific antibodies

Using peptides specific to either type I or type II protein, or an epitope common to both proteins, several type-specific or pan-IMPDH antibodies were generated. A titration of recombinant type I and type II protein was used to determine the specificity and sensitivity of each antibody (Fig. 5). Both antibodies detect a 56 kDa protein as expected. The type I antibody is observed to be 4-fold more sensitive; it can detect amounts as low as 5 ng of recombinant type I protein whereas the type II antibody only recognizes 20 ng or higher levels of type II protein with the methods used. IMPDH type I antibody can cross-react with IMPDH type II recombinant protein but only at a 20-fold higher concentration of type II protein than type I protein and, hence, is type I-selective. The pan-IMPDH antibody detects both type I and type II recombinant proteins, perhaps with a 2-fold higher sensitivity for the type I protein as compared with the type II protein.

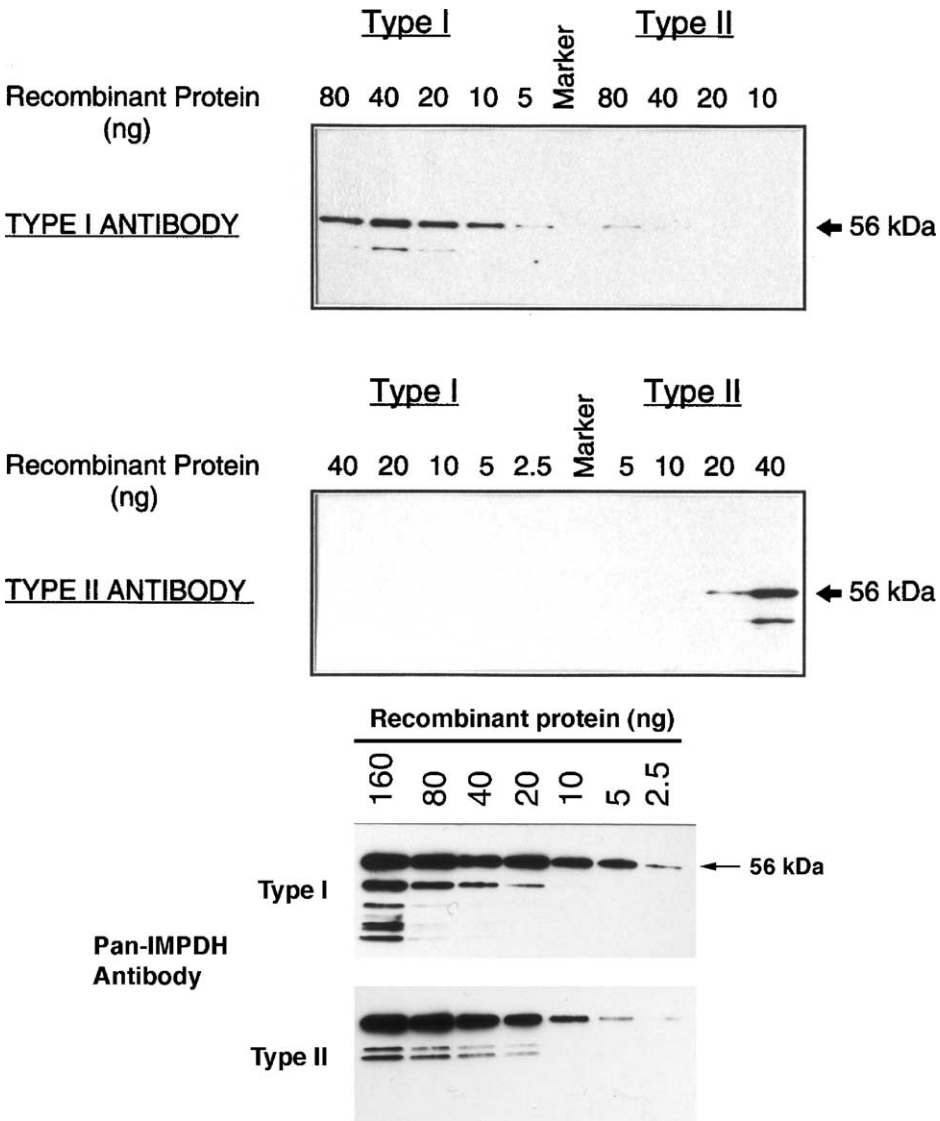

Fig. 5. Specificity of IMPDH type I and II antibodies. Affinity-purified antibodies specific to IMPDH type I (top panel), type II (middle panel), or both (lower panel), proteins were hybridized with recombinant purified type I and type II proteins. The concentration of recombinant protein, loaded per lane, in nanograms (ng), is indicated at the top of each lane.

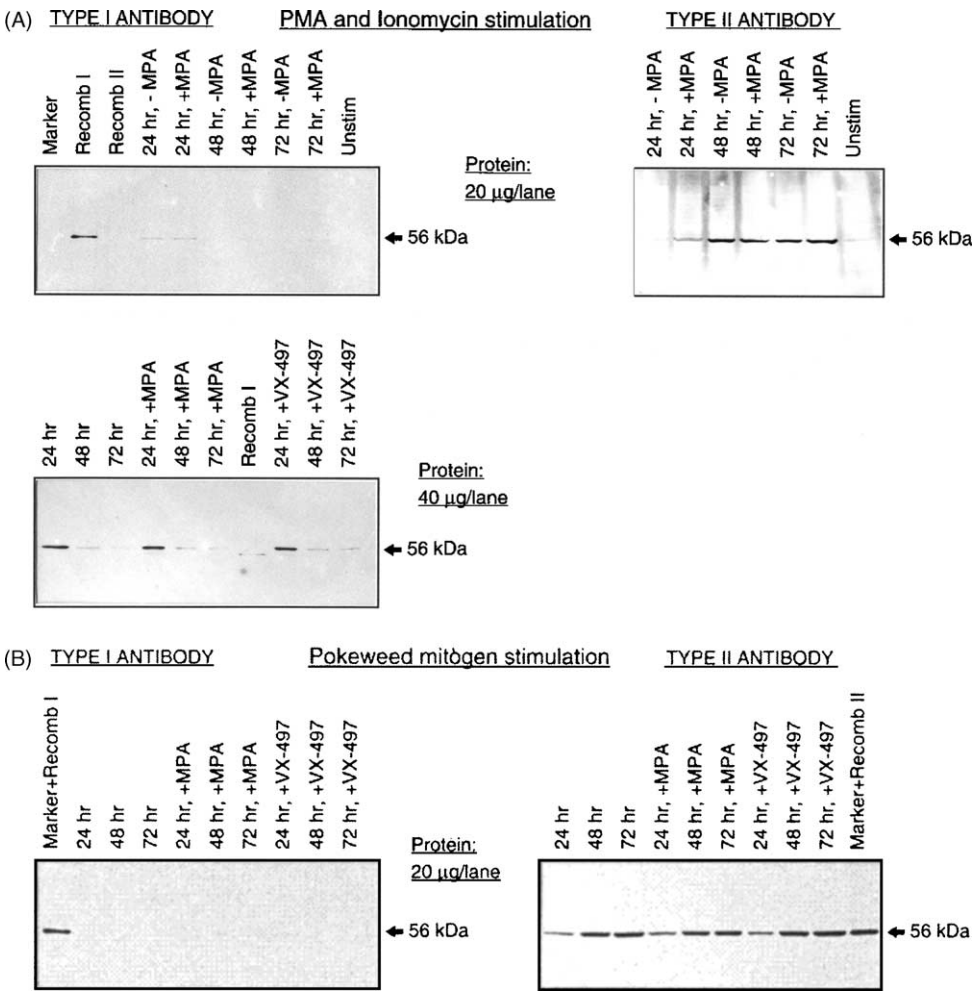

Fig. 6. IMPDH type II protein is more abundant in human lymphocytes. Total protein was isolated from unstimulated PBMCs or PBMCs stimulated with 10 ng/mL PMA and 250 ng/mL Ionomycin (A), or 25 µg/mL pokeweed mitogen (B), for 24, 48, or 72 hr. DMSO (0.1%), or 1 µM MPA or VX-497 were included during the mitogen-stimulation as indicated. The blots were hybridized with IMPDH type I- or type II-specific antibodies. Recombinant type I and type II IMPDH proteins were included to confirm the specificity of the bands. Although the type I antibody demonstrates greater sensitivity than the type II-specific antibody, a 2-fold higher concentration of protein (40 µg total protein loaded per lane in 'A' lower panel), was needed to observe IMPDH type I protein as compared with the type II protein (20 µg total protein loaded per lane in the 'A' top panel).

3.6. Determination of IMPDH type I and type II protein levels in PBMCs

A single band of 56 kDa was also observed in human PBMCs stimulated with PMA and Ionomycin for 24, 48 or 72 hr (Fig. 6A). Neither type I nor type II IMPDH proteins were detected in unstimulated lymphocytes. However, both type I and type II proteins are upregulated in response to mitogenic stimulation. Induction of type I protein is observed 24 hr after stimulation with the protein levels decreasing by 48 and 72 hr. In contrast, type II protein appears within 24 hr but continues to increase and persist up to 72 hr. The type I and type II protein levels are consistent with the mRNA levels observed for both isoforms: type I mRNA levels appear maximal at 24 hr post-stimulation with a decline obvious by 48 and 72 hr, while the type II mRNA levels appear high at both 24 and 48 hr with significant levels still apparent at 72 hr (see Fig. 2). Therefore, both type I and type II IMPDH isoforms are upregulated in human PBMCs in response to T or B cell mitogens, albeit with slightly different kinetics. Overall, IMPDH type II protein appeared to be more abundant in PBMCs stimulated with PMA and Ionomycin, or PWM (Fig. 6B).

3.7. Effect of MPA or VX-497 treatment on IMPDH protein levels

PBMCs stimulated with PMA and Ionomycin or PWM were treated with 1 µM MPA or VX-497 for time periods up to 72 hr (Fig. 6A and B). Neither compound had any effect on the level of type I or type II protein. Higher steady-state levels of type II protein were observed in some cell lines such as Jurkat T cells, whereas higher levels of type I protein were observed in WI38 fibroblast cells (Fig. 7) demonstrating that either isoform can be upregulated in spontaneously proliferating cells. Treatment with 1 µM MPA or 1 µM VX-497 for 72 hr had no significant

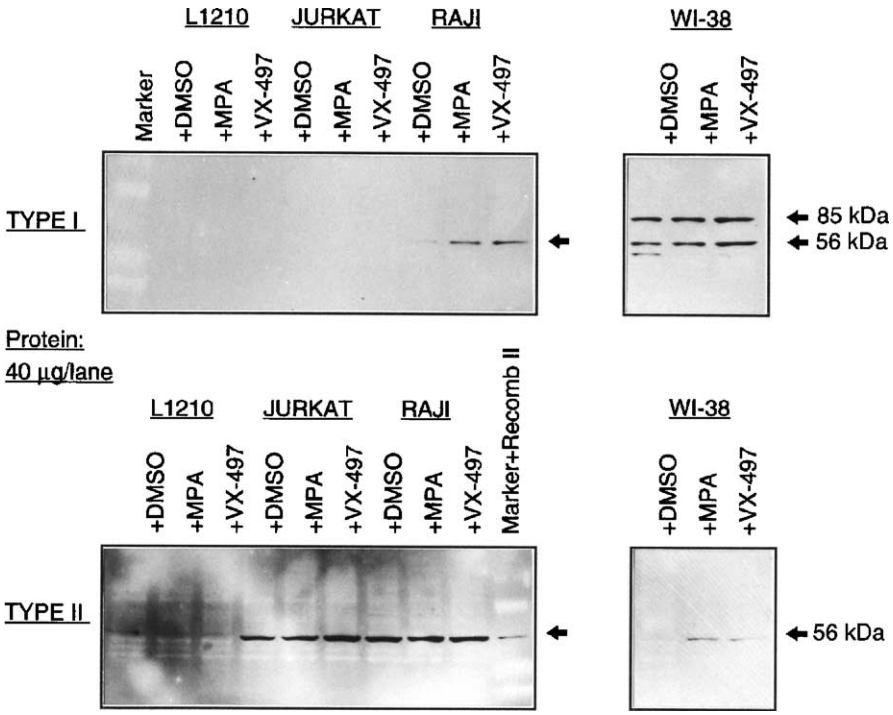

Fig. 7. Treatment of lymphoid or fibroblast cell lines with MPA or VX-497 does not alter IMPDH protein levels. Total protein was isolated from unstimulated L1210, Jurkat, Raji, and WI38 cells treated with 0.1% DMSO, 1 µM MPA or 1 µM VX-497 for 72 hr. The blots were hybridized with IMPDH type I (top panel), or IMPDH type II (lower panel), selective antibodies.

effect on IMPDH type I or type II levels in cell lines (Fig. 7).

4. Discussion

IMPDH has been evaluated as an immunosuppressive, anti-tumor, and anti-viral drug target for more than a decade. The presence of two highly homologous IMPDH enzymes raises the question of their relative importance and contribution to intracellular processes leading to various disease states. These studies were initiated to determine the need for isoform-specific IMPDH inhibitors by understanding the regulation and expression patterns of the two IMPDH genes. Our results indicate that the stimulation of normal human lymphocytes leads to the induction of both type I and type II mRNAs and protein suggesting that both isoforms play a role in lymphocyte proliferation. Based on the previous studies of Dayton *et al.* [21], and the results presented in this study, inhibition of both IMPDH isoforms appears desirable for blocking the proliferation of lymphocytes. Since MPA inhibits both IMPDH isoforms with similar potency, long-term use of MMF as an immunosuppressive agent suggests that it is safe to inhibit both isoforms. In a compassionate-care trial for psoriasis, where patients were dosed with MPA for up to 13 years, no adverse clinical effects specifically linked to the inhibition of type I isoform were reported [23]. Presumably this is because most cell types can utilize the alternate salvage nucleotide synthesis pathway.

Previous conclusions regarding the specific up-regulation of type II IMPDH activity in replicating cells were based on studies performed mostly with transformed or neoplastic cells. We elected to use normal human lymphocytes to determine the status of the two isozymes in unstimulated, quiescent lymphocytes and compare it with that in normal lymphocytes stimulated with mitogens. It was possible to perform a time-course of induction to follow the kinetics of mRNA and protein expression since fresh PBMCs represent a near-homogenous, synchronous population of >95% cells that do not divide until they are stimulated. PBMCs, stimulated with mitogens such as PMA and Ionomycin, start proliferating approximately 36–48 hr after stimulation, and continue to proliferate for another 24–36 hr. In these studies, both isoforms were induced within the first 48 hr, with increased protein levels detected within the 48 hr. Induction of both IMPDH mRNA species was observed with stimulation with different mitogens, PMA and Ionomycin, as well as with PWM.

While a single transcript was observed for IMPDH type I mRNA in most tissue and cell types, three type I mRNA species were consistently observed in human PBMCs. Dayton *et al.* [21] also reported two transcripts for IMPDH type I mRNA in human PBMCs.. These correspond to the 3.5 and 2.5 kb mRNA bands in this study. The presence of multiple mRNA species may be due to the utilization of three alternative promoters that have been mapped upstream of the IMPDH type I gene [24] resulting in a complex transcriptional regulation of the type I gene in human lymphocytes. The transcriptional regulation of the

type II gene appears to be more dependent on protein–protein interactions or on post-translational modifications of transcription factors bound to the different promoter sites since they are occupied in unstimulated as well as stimulated cells [25,26].

To date, there have been no reports on the expression or regulation of IMPDH type I or type II proteins. Dayton *et al.* [21] used isoelectric focusing (IEF) but were unable to distinguish between the two isoforms. Hence, no correlation has been made between specific levels of type I or II protein with the replicative or differentiation fate of cells. Using type-selective antibodies, this is the first study to demonstrate the induction of both proteins in response to stimulation, and the lack of any feedback regulation by IMPDH inhibitors on either isoform. While gene knockout experiments have also indicated a non-redundant role for each IMPDH gene [7,8], it would be useful to determine the function of each isozyme in adult lymphocytes using lymphocyte-specific (RAG), or other tissue-specific knockout approaches.

The effect of IMPDH inhibitors on regulating IMPDH enzyme activity over prolonged time-periods has been examined previously. Increased IMPDH enzyme activity has been reported in erythrocytes of ribavirin-treated patients [27] or organ transplant patients taking MMF [28]. However, no increase in IMPDH activity was observed in patient lymphocytes [28]. We believe that the relevant compartment for monitoring IMPDH activity is the lymphocytes rather than erythrocytes since lymphocytes are primarily responsible for the induction of tolerance or rejection of the transplanted organ. Furthermore, if the induction of IMPDH activity in the erythrocyte compartment were clinically relevant, one would expect to see the efficacy of MMF decrease over time, with patients developing resistance to MMF. No instances of resistance, or dose-increments to compensate for resistance to MMF, have thus far been reported in organ transplant or psoriasis patients despite their use of MMF for more than 5 years. Hence, based on the past clinical experience with MMF, the elevated IMPDH enzyme activity observed in erythrocytes does not translate into reduced efficacy of MMF or stimulation of lymphocyte proliferation. As shown in this study, IMPDH inhibitors do not appear to alter the regulation of IMPDH type I or type II mRNA or protein levels, or induce IMPDH activity [28] in lymphocytes.

In conclusion, our results demonstrate that both IMPDH isoforms are regulated at the transcriptional level, whether altering the induction of mRNA or its stability. Both genes are expressed in most tissues with partially overlapping patterns of expression. The level of expression of type I and type II mRNA and protein differed among different cell lines. Furthermore, prolonged treatment of human PBMCs or cell lines with uncompetitive IMPDH inhibitors such as VX-497 or MPA does not result in significant alteration of either type I or type II mRNA or protein levels. These results suggest that both IMPDH isoforms should be considered targets for immunosuppressive therapy and that chronic use of VX-497 for the treatment of autoimmune and other indications would not lead to an induction of IMPDH activity in target tissues.

Acknowledgments

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